

Applicants: David S. Lawrence and Biao Xi
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Amendments to the Specification:

Please amend paragraph [0030] as indicated below.

[0030] *Plasmid construction.* The wild type human fibroblast cell line GM02037 (Coriell Cell Repositories, NJ) was used as the DNA template for *atm* promoter amplification. In brief, the Qiagen Blood & Cell Culture DNA Mini kit was used to purify genomic DNA. ATM gene promoter primers, which were used to amplify the desired fragment DNA, were designed based on the sequence in Genbank. The forward primer sequence employed was GATCAAAACCACAGCAGGAAC (SEQ ID NO:1) and the reverse primer was GCCACGGGAGGAGGCGAG (SEQ ID NO:2). PCR was carried out using the Roche Expand High Fidelity PCR system. The amplified *atm* promoter region was then cloned into the TOPO2.1 PCR vector (Invitrogen) for sequencing verification and subsequently subcloned into the promoterless plasmid PA3Luc. Firefly luciferase cDNA was mutated (QUIKCHANGE® ~~QuikChange~~ Site-Directed Mutagenesis Kit) in the desired region to furnish the three stop codons, as shown as Figure 1(C). The CMV driven Renilla luciferase plasmid was purchased from Promega as the internal control for the DUAL LUCIFERASE® ~~dual luciferase~~ assay.

Please amend paragraph [0032] as indicated below.

[0032] Transfections were performed using GENEJAMMER® ~~GeneJammer~~ reagent (Stratagene) based on the protocol from the manufacturer. In brief, approximately 15 x 10⁵ HEK293T cells were seeded into white 96 well plates (Corning) and grown for less than 24 hours. Cells were transfected with 0.6 µL of GENEJAMMER® ~~GeneJammer~~ and 0.1 µg plasmids. In the DUAL LUCIFERASE® ~~dual luciferase~~ assay, the plasmid ratio of

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ATM promoter-driven firefly luciferase to CMV promoter-driven Renilla luciferase was 30:1. In the DUAL LUCIFERASE® ~~dual-luciferase~~ experiments that utilized the stop codon-containing firefly luciferase genes, the ratio was 40:1. On the second day after transfection, the medium in each well was changed, and the promoter and/or nonsense-suppressing agents added to the wells for a 24 hour incubation period. The cells were subsequently lysed and promoter activity/nonsense suppression efficiency then assessed via the DUAL LUCIFERASE® ~~dual-luciferase~~ assay.

Please amend paragraph [0033] as indicated below.

[0033] DUAL LUCIFERASE® ~~Dual-luciferase~~ assay. The DUAL LUCIFERASE® ~~dual-luciferase~~ kit was purchased from Promega and the protocol employed was conducted according to the directions supplied by the manufacturer. In brief, cells were washed with PBS and lysates were prepared using passive lysis buffer. Luminescence was determined with a Molecular Devices Lmax 96 well plate luminometer. Light emission was measured after each of the 100 μ L luminescence substrates and/or stop solutions were injected. ATM promoter activity was calculated by the ratio of relative light units of flash (firefly) to glow (renilla) luminescence in each well, which was compared with control wells (cultured only in medium with plasmid but no drug treatment) in each plate. In the case of the stop codon suppression studies, controls also included cells containing the plasmids without the inserted stop codon, which were treated with nonsense suppressing and/or promoter activating agents. All individual treatments in a given experiment were performed in triplicate and all experiments repeated 3 - 10 times.

Please amend paragraph [0035] as indicated below.

[0035] *mRNA Quantitation.* Single-stranded cDNAs were synthesized by reverse transcription, using a First Strand cDNA Synthesis kit (Invitrogen), and then used as a template in polymerase chain reactions. ATM mRNA was primarily quantified using real time polymerase chain reaction (PCR). In brief, cells were treated with the desired drugs or combinations thereof under conditions identical to those described above for the DUAL LUCIFERASE® ~~dual-luciferase~~ assay. Cells were lysed and RNA extracted with the TRIZOL® ~~TRIzol~~ reagent (Invitrogen). Approximately 1 μ g of total RNA in each sample was subjected to cDNA synthesis using the First Strand cDNA Synthesis kit (Invitrogen). In the real time PCR reaction, the FAM labeled MGB probe, CCAGCTATTTGGTTTGAG (SEQ ID NO:3), was designed by the PRIMER EXPRESS® ~~Primer-Express~~ software from Applied Biosystems (AB) and synthesized by AB. The human β -actin MGB probes, VIC or FAM fluorescently labeled, were purchased from AB. All other reaction reagents were purchased from AB. PCR reactions were performed using the ABI PRISM 7000 Sequence Detection System in triplicate. Some samples were also tested on the ABI 7700 Sequence Detection System for verification and essentially identical results were obtained with both real time PCR instruments. All collected data were compared with a β -actin control. In addition to real time PCR, the branched chain DNA (bDNA) RNA quantitation method was employed as well. Briefly, the QuantiGene kit as well as the customer designed *atm* probe sets were purchased from Genospectra. The latter probes target the same region on the *atm* message RNA region as the real time PCR probes. The cells were treated as described above for the DUAL LUCIFERASE® ~~dual-luciferase~~ assay. Cell lysates were transferred into a capture plate and *atm* mRNA allowed to hybridize with the probe set at 53 °C overnight. After 16-20 hours, the signal was amplified and subsequently read using

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a Molecular Devices Lmax luminometer. The internal human β -actin probe sets were purchased from Genospectra. All experiments were performed in triplicate.

Please amend Table 2 as indicated below.

Table 2. Fold-Increase in Stop Codon Read-through in 293T Cells Treated with Aminoglycoside Alone or Aminoglycoside in Combination with Promoter Activating Agent.

Protocol	Fold-increase (read-through) ^a		
	opal	amber	ochre
Geneticin	6.5 ± 0.8	14.9 ± 1.9	7.9 ± 3.0
Gentamicin	8.7 ± 1.2	8.2 ± 1.8	6.7 ± 1.5
Ofloxacin/Gentamicin	14.3 ± 1.2	17.5 ± 1.9	9.6 ± 2.1
Thioguanine/Gentamicin	32.1 ± 6.2	29.2 ± 5.9	18.3 ± 2.3
Ofloxacin/Geneticin	14.6 ± 3.5	21.1 ± 1.1	7.1 ± 0.4

^aAs measured by the DUAL LUCIFERASE® dual-luciferase assay (in triplicate) and relative to untreated 293T cells transfected with the indicated stop codon-containing gene.